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The specialised structure of crypt epithelium in the human palatine tonsil and its functional significance

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ABSTRACT

Material from 25 human palatine tonsils was studied by light microscopy, immunocytochemistry, scanning and transmission electron microscopy. Special attention was focused on the structure of the epithelium lining the tonsillar crypts in the context of its ascribed immunological functions. This epithelium was not uniform and contained patches of stratified squamous nonkeratinising epithelium and patches of reticulated sponge-like epithelium. The degree of reticulation of the epithelial cells and the infiltration of nonepithelial cells varied. Reticulated patches were associated with disruptions in the continuity of basement membrane, and often also with desquamation of the upper cell layers, and contained numerous small blood vessels. The epithelial cells showed considerable variation in their morphology when surrounded by infiltrating cells. The rearrangement of their cytoskeleton and redistribution of desmosomal contacts indicate the responsiveness and dynamic nature of such epithelium. Cytoplasmic glycogen granules, located in the upper strata, suggest the possibility of energy-demanding functions such as absorption and secretion. The numerous membrane-coating granules may have contributed to cell membrane thickening and possibly also to tonsillar mucosal protection. Some areas contained a few keratohyalin granules but there was little evidence of keratinisation. The presence, and sometimes the predominance, of nonepithelial cells was characteristic of the reticulated epithelium. T and B cells often infiltrated the whole epithelial thickness, and many plasma cells were located around intraepithelial vessels, while macrophages and interdigitating cells showed a patchy distribution. It is proposed that the major functions of the reticulated epithelium are: (1) to provide a favourable environment for the intimate contact between the effector cells of immune responses; (2) to facilitate direct transport of antigens; (3) to synthesise the secretory component continually; and (4) to contain a pool of immunoglobulins. Thus the reticulated epithelium lining the tonsillar crypts represents a specialised compartment, important in the immunological functions of the tonsil as a whole.

Key words: Lymphoid tissue; reticulated cells; mucosal protection.

INTRODUCTION

The epithelium of the human palatine tonsil is a site of interest because it overlies a mass of lymphoid tissue that forms a first line of defence in protecting the oropharyngeal isthmus, and as such is constantly exposed both to airborne and alimentary antigens. The surface area of the tonsillar epithelium available for this defensive role is maximised by the 10–30 blind-ending crypts and their rami (Kassay & Sandor, 1962; Oláh, 1978), extending through the full thickness of the tonsil almost to reach its hemicapsule (Kassay, 1938; Fioretti, 1957).

The epithelium lining the crypts is a highly modified form of the stratified squamous epithelium that covers the oropharyngeal surface of the tonsil. As early as 1884, Stöhr recognised that crypt epithelium was usually infiltrated by lymphocytes. This observation was later confirmed by Barnes (1923) and Kahler (1933), the latter proposing that the function of the palatine tonsil was 'protection and defence'.

Fioretti (1957) termed the mixing of lymphoid and epithelial cells in the crypt epithelium 'simbiosi linfoepitheliale' (the lymphoepithelial symbiosis). Hellman (1932) and Koburg (1967) likened the network of epithelial cells to a sponge 'der epithelialer Schwammkörper', which holds the lymphoid cells in its interstices. In 1978 Oláh termed this specialised

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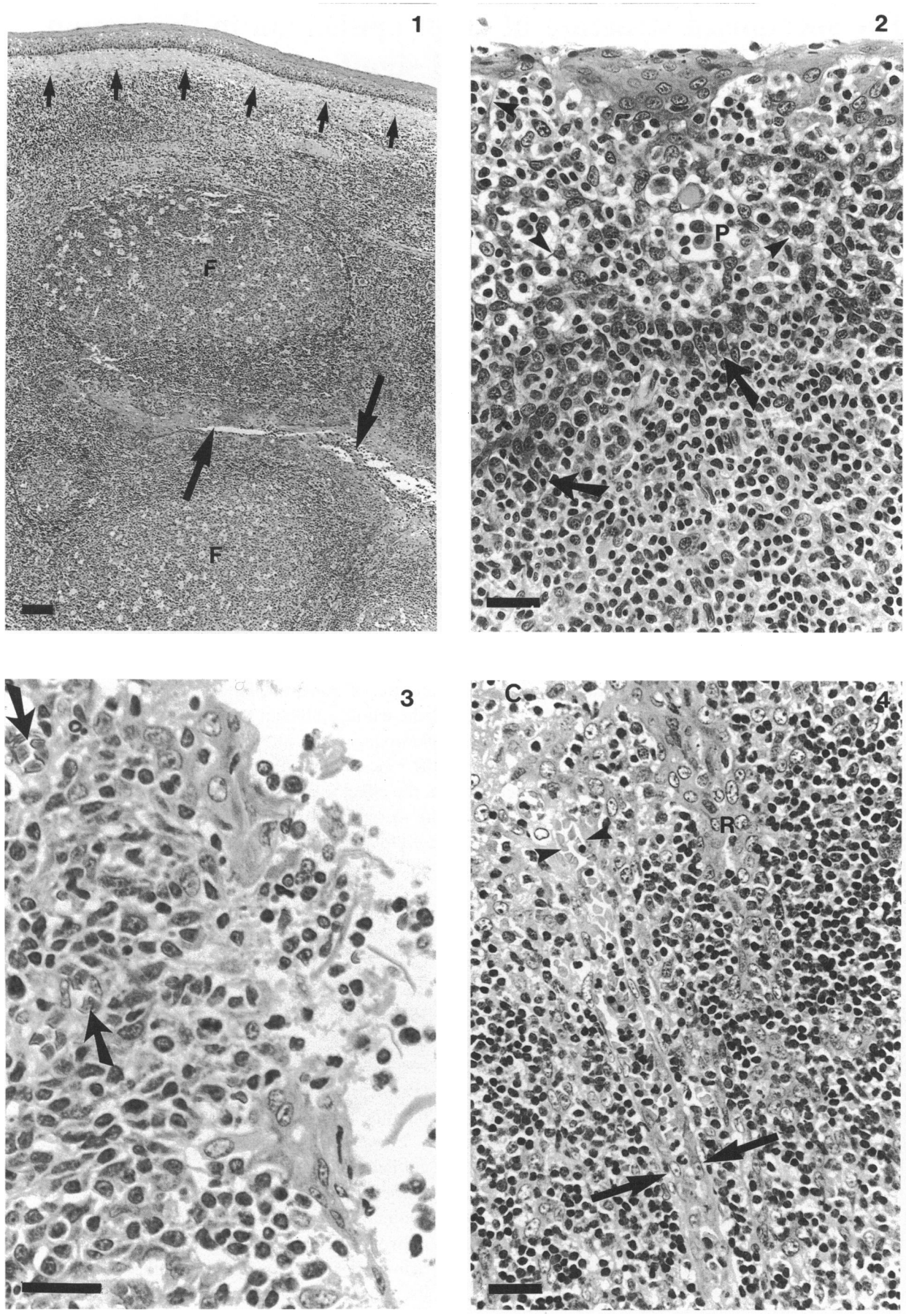


Figs 1–4. For legend see opposite.

crypt epithelium 'reticular epithelium' and found that it lined most, though not all, of the cryptal surfaces. The nonepithelial elements identified in the reticulated epithelium include lymphoid cells, numerous antigen presenting cells (Yamamoto et al. 1988; Perry & Mustafa, 1992) and a network of small blood vessels (Fujihara et al. 1988, 1989; Perry et al. 1992*a*, *b*).

The significance of this specialised epithelium has been highlighted by Korsrud & Brandtzaeg (1981*a*, *b*), Brandtzaeg (1988) and Brandtzaeg & Halstensen (1992) following their comprehensive immunological studies on human palatine and nasopharyngeal tonsils. They were the first to propose that in addition to the germinal centre, mantle zone and interfollicular regions, the tonsillar crypt epithelium functions as an additional lymphoid compartment by contributing to the production of immunocytes and to the protection of the mucosal surface (Korsrud & Brandtzaeg, 1981*b*; Cortesina et al. 1992; Kataura et al. 1992).

The aim of the present study was to investigate the specialised structure of the crypt epithelium in relation to its ascribed immunological function, using light microscopy, and scanning and transmission electron microscopy.

MATERIALS AND METHODS

Human palatine tonsils were obtained randomly from 26 children (17 boys and 9 girls) undergoing tonsillectomy. The youngest child was aged 23 months, and the oldest was 14 y 4 months at the time of the operation. Preoperative diagnoses ranged from recurrent tonsillitis and upper respiratory tract infection, with or without otitis media, to the non-inflammatory condition of nasal obstruction with nocturnal apnoea. None of the patients suffered from acute tonsillitis or had received a course of antibiotics in the immediate preoperative period.

Light microscopy

Architecture of the epithelia. Tonsillar samples, containing both oropharyngeal and crypt epithelia, were fixed in 10% formol saline and processed for paraffin wax embedding. Subsequently, 2–4 μm sections were obtained, which were stained with haematoxylin and eosin, Heidenhain's azan and Gordon and Sweet's stain for reticulin (Bancroft & Cook, 1984). The latter two were used to demonstrate the connective tissue components of basement membranes of both epithelia and blood vessels.

Cellular inclusions. Sections were also stained using the periodic acid-Schiff technique (PAS) for examining glycogen distribution; performic acid-methylene blue (PAMB) for keratin, with human skin as a control; Gram's stain for keratohyalin; Alcian blue (range of pH between 1 and 2) and Csaba's stain (acetate buffer pH 1.42) for mast cells; and Giemsa's stain for aiding identification of plasma cells. All methods were carried out according to Bancroft & Cook (1984).

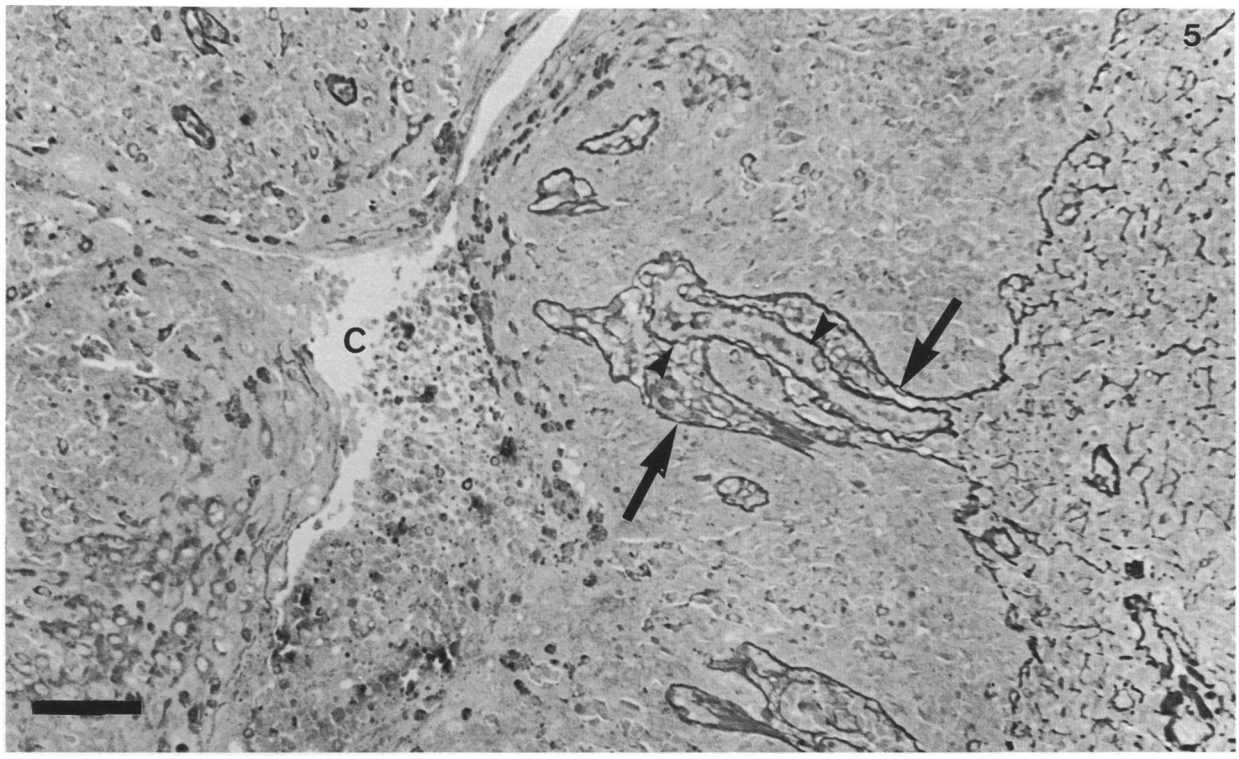
Immunocytochemistry. Small pieces ($\sim 5\ mm^3$) of fresh tissue were oriented using the binocular microscope to ensure the presence of crypt epithelium in subsequent sectioning. They were then embedded in Tissue Tek and frozen in melting isopentane precooled in liquid nitrogen. Frozen sections (4 μm) were obtained, which together with the 2 μm paraffin sections, were used for immunocytochemistry. The antibodies applied were CD3 (anti-LEU-4) (Becton Dickinson Immunocytometry Systems) for T cells; MB2 (Euro-Diagnostic BV, Holland) for B cells; and S-100, Cow S-100 (Dakopatts, Denmark) for macrophage/dendritic cells. The direct and indirect routine peroxidase-antiperoxidase (PAP) technique was used to visualise the reactions. Incubation with primary antibodies was carried out for 1 h in a wet chamber at room temperature. Anti-LEU-4 and MB2 antibodies were used in 1/10 dilution and S-100 in 1/75 dilution. Secondary antibodies used were 1/20 dilution of either rabbit antimouse or swine antirabbit conjugated antiserum, applied to the sections for 1 h or 30 min respectively, in a wet chamber at room temperature. Positive labelling was identified as distinctive brown reaction products visible with a light microscope. The PAP method was applied to control sections with the omission of primary antibodies or

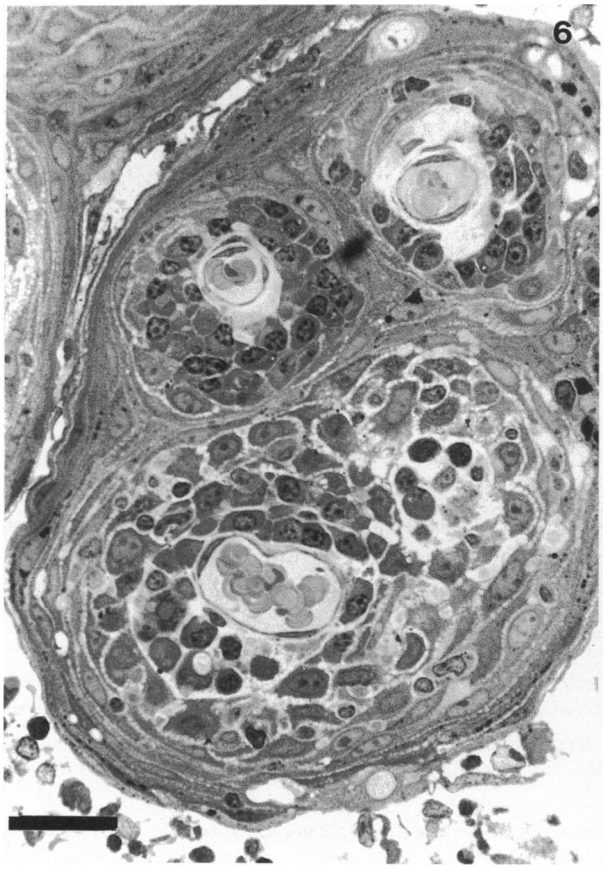
Fig. 1. The oropharyngeal surface of the palatine tonsil (top) is covered with stratified squamous nonkeratinising epithelium, underlined by a connective tissue band (short arrows). Transected tonsillar crypt (long arrows). F, lymphoid follicles. Haematoxylin and eosin. Bar, 100 μm.

Fig. 2. A patch of reticulated epithelium (upper half) covered with a thin layer of squamous cells. Note distorted epithelial cells with attenuated cytoplasmic processes (arrowheads) and an indistinct boundary between the epithelium and the underlying lymphoid tissue (arrows). P, intraepithelial plasma cells. Haematoxylin and eosin. Bar, 25 μm.

Fig. 3. Reticulated crypt epithelium containing many infiltrating nonepithelial cells and transected capillaries (arrows). The epithelial surface (right) shows a 'fountain-like' disruption. Haematoxylin and eosin. Bar, 25 μm.

Fig. 4. Heavily reticulated patch of crypt epithelium containing a longitudinally sectioned vessel lying in parallel to and below an obliterated ramus of the crypt (R). The endothelium lining the vessel is low (arrowheads) near the crypt lumen (C) and high (arrows) in the segment of the HEV. Haematoxylin and eosin. Bar, 25 μm.





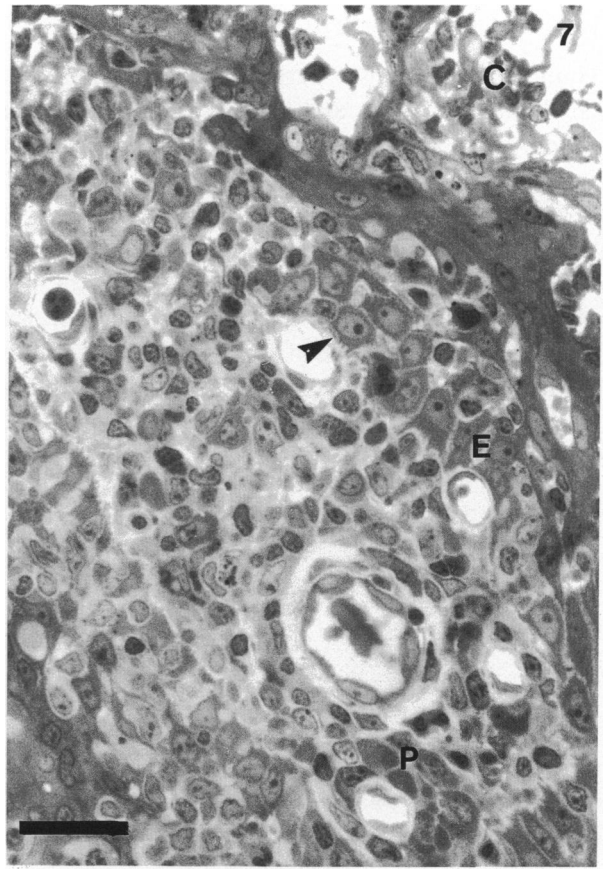


Fig. 5. Finger-like projections of subepithelial connective tissue sectioned in both longitudinal and transverse planes. They are located in the epithelium and contain inner blank outlines (arrowheads), indicating the basement membrane of blood vessels, surrounded by outer black outlines (arrows), indicating the basement membrane of the epithelium. C, crypt lumen. Gordon and Sweet. Bar, 100 μm.

substitution of the primary antibody by an irrelevant antibody.

Scanning and transmission electron microcopy (SEM and TEM)

Samples containing crypt epithelium were prepared and oriented using a dissecting microscope, fixed in Karnovsky's fluid at 4 °C for 4 h and transferred to 0.1 M sodium cacodylate buffer at 4 °C for a further 12 to 16 h. They were postfixed in 1 % osmium tetroxide in 0.1 M sodium cacodylate buffer at 4 °C for 2 h, and dehydrated in graded alcohols.

The TEM samples were placed in propylene oxide at room temperature for 20 min and embedded in Epon 812 resin. Semithin sections (1 μm) were cut and stained using methylene blue/azur II (Richardson et al. 1960). Ultrathin sections (70 nm) were stained using uranyl acetate in methanol, counterstained with lead citrate, and examined using a Hitachi 300 electron microscope.

The SEM samples were placed in ascending grades of acetone, critical point dried, mounted onto stubs, sputter coated with gold and examined using an Hitachi S 520 scanning electron microscope.

RESULTS

Light microscopy

Architecture of the epithelia

The oropharyngeal aspect of the palatine tonsil was covered either with nonkeratinised or parakeratinised stratified squamous epithelium approximately 15–20 cells thick, and contained a few nonepithelial cells. This epithelium was supported by a layer of collagenous connective tissue approximately 10–40 μm thick, which carried a network of blood vessels and separated it from the underlying lymphoid tissue (Fig. 1).

The crypts communicated with the oropharynx either directly, through a crypt mouth, or indirectly, through a lacuna, an area common to several crypts. Some crypts were tubular with a few lateral ramifications, others ramified in a complex dendritic manner. Many crypts extended almost to the hemicapsule of the tonsil and their lumina were either empty, or filled with desquamated cells and debris, in some instances occluding them altogether.

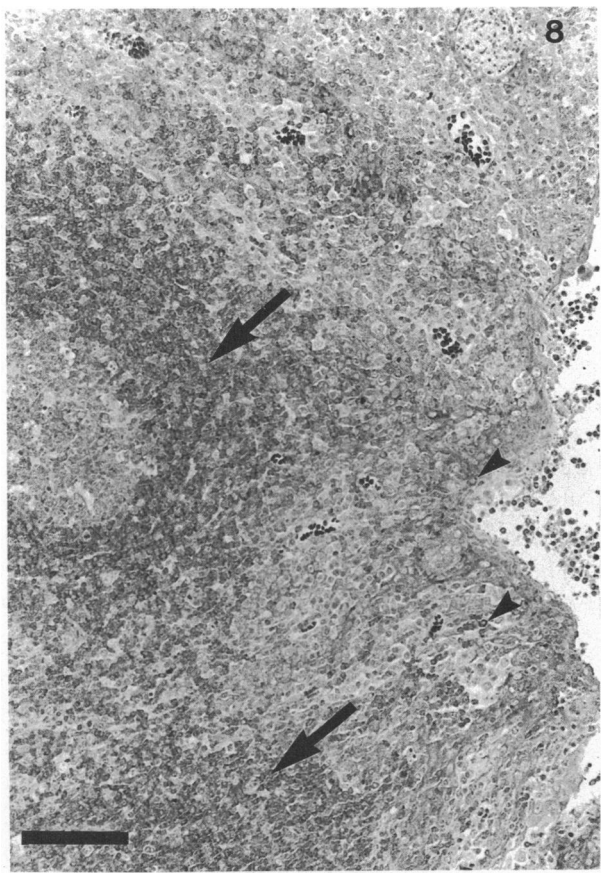
The epithelium lining the crypts was not uniform. There were patches of stratified squamous and patches of reticulated epithelium (Fig. 2). The stratified squamous epithelium was of a similar structure to that covering the oropharyngeal surface except that it did not possess a thick underlying layer of collagenous connective tissue, it usually directly overlay lymphoid tissue, often it contained isolated infiltrating nonepithelial cells and it was thinner (approximately 1–10 cells thick) than the stratified squamous epithelium covering the oropharyngeal surface (Figs 1, 2).

The reticulated epithelium was usually divisible into 3 layers: the basal layer, the intermediate or prickle cell layer, and the superficial or surface layer (Fig. 2). The basal cells maintained an orientation perpendicular to the basement membrane. However, this layer was often incomplete, and its supporting basement membrane disrupted. No mitoses were observed. In contrast to the basal cells, the prickle cells were usually distorted, and separated from one another by the invading nonepithelial cells (Fig. 2). The elongated extensions of the prickle cells formed a network connecting the basal and superficial strata (Fig. 2). Finally, the surface of the reticulated epithelium was either covered by one or more layers of horizontally oriented squamous cells (Fig. 2), or it was disrupted in those places where nonepithelial cells passed into the crypt lumen (Fig. 3). Most of the infiltrating nonepithelial cells were small lymphocytes, although lymphoblasts, plasma cells, macrophages, occasional polymorphonuclear leucocytes and extravasal erythrocytes were also seen (Figs 2, 3).

The reticulated epithelium usually lacked a subepithelial layer of collagenous connective tissue (Figs 1, 2), which was otherwise reduced to a few fine discontinuous fibres. This epithelium followed the convex contours of the underlying follicles and plunged deeply into the interfollicular areas; it either rested directly on, or actually contained, the lymphocytic caps (Fig. 1).

Fig. 6. Transected nodular projection of tonsillar epithelium showing lumina of 4 vessels lined with low endothelium. Three of these vessels are surrounded by concentrically arranged plasma cells, external to them are layers of epithelial cells. Semithin section, methylene blue/azur II. Bar, 25 μm.

Fig. 7. Sectioned vessels in reticulated crypt epithelium. Their walls are surrounded by nonepithelial cells, such as plasma cells (P), or by epithelial cells (E). These extend several rootlet-like projections towards the endothelial basement membrane (arrowhead). The crypt lumen (C) contains desquamating cells. Semithin section, methylene blue/azur II. Bar, 25 μm.



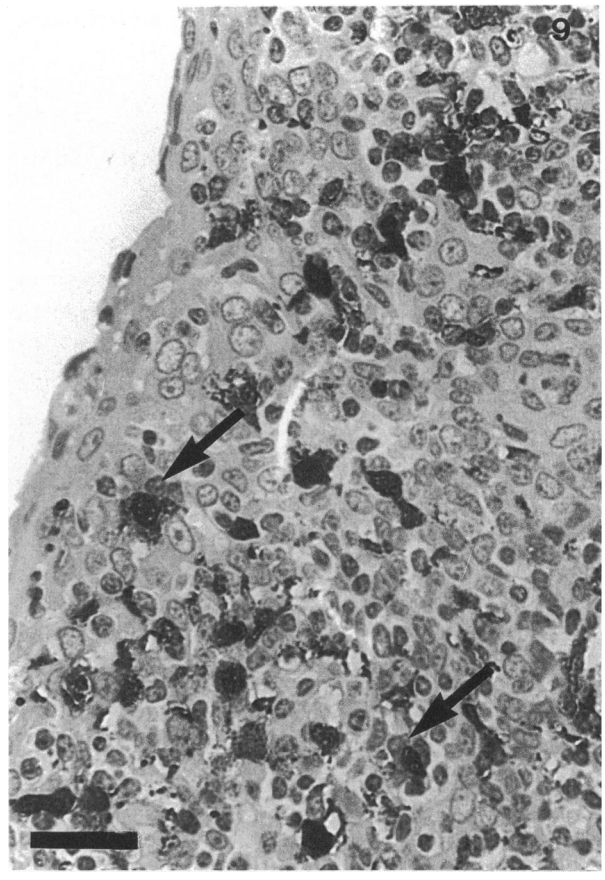


Fig. 8. The greatest accumulation of B cells is in the follicular caps (arrows). Some are also in the reticulated epithelium (arrowheads) and a few are among the crypt contents. The scattered groups of dark cells (nonspecific staining) are intraluminal erythrocytes. MB2 antibody on paraffin section. Bar, 100 μm.

Fig. 9. Reticulated crypt epithelium showing large darkly stained S-100 positive cells surrounded by lymphoid cells (arrows). S-100 antibody on paraffin section. Bar, 25 μm.

The degree of reticulation varied both in the size and the number of reticulated epithelial patches in a crypt (Figs 3, 4). Furthermore, within a single patch, the density of the infiltrating nonepithelial cells was highly variable. These patches were apparently randomly distributed and were seen overlying either the follicles or the interfollicular areas. However, they were always found to be associated with disruptions in the continuity of the basement membrane, and often with desquamation of the superficial cell layers (Figs 1–3). When the epithelium was heavily reticulated (Fig. 4) it was difficult to define the junction between it and the underlying lymphoid tissue. The basement membrane formed neither a continuous nor a disrupted layer, but was found as isolated fragments of PAS positive material. The adjacent basal cells were arranged in small islands, widely separated by lymphoid tissue. Similarly, the prickle cells were seen in small, apparently isolated clumps surrounded by numerous nonepithelial cells, rather than forming a network connecting the basal and superficial epithelial layers (Fig. 4).

Small blood vessels were seen both below the epithelium and extending throughout its thickness. Many were located within finger-like projections of subepithelial connective tissue and were surrounded by a perivascular sleeve containing numerous plasma cells (Figs 5, 6). In addition to these finger-like projections, many capillaries (6–8 μm in diameter) appeared to be in direct contact with the epithelial cells (Figs 3, 7) and in some sections, lymphocytes were found diapedesing through their walls.

Although high endothelial venules (HEVs) were mostly situated in the subepithelial interfollicular areas, they were also found within the lower portion of the reticulated epithelium. However, in the areas of heaviest reticulation, where the epithelium was modified to its greatest degree, HEVs extended throughout its thickness (Fig. 4).

Cellular inclusions

Glycogen was identified as PAS positive intracellular granular material. The amount of glycogen within

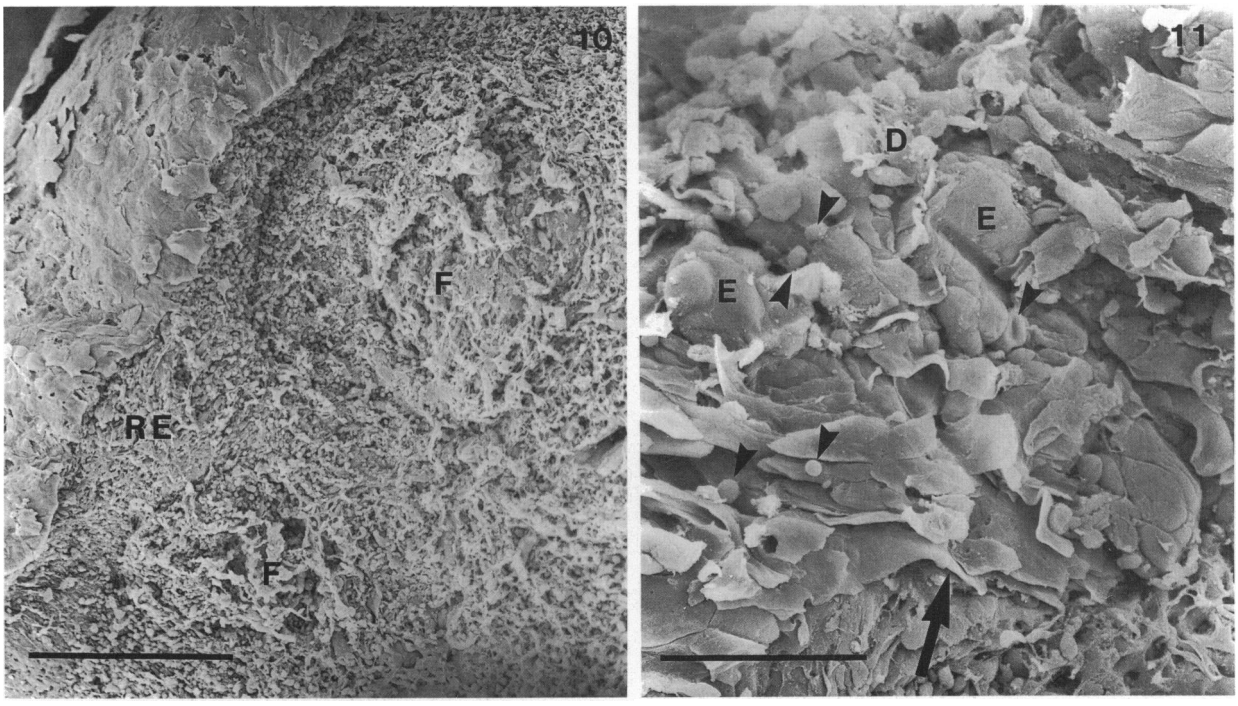


Fig. 10. Crypt surface with a crust-like layer of epithelial squames (left) of the reticulated epithelium (RE). Note the 2 underlying lymphoid follicles (F). SEM. Bar, 50 μm.

Fig. 11. Surface of crypt epithelium containing a mixture of epithelial cells (E), nonepithelial cells (arrowheads) and cellular debris (D). This cover is discontinuous and only one to two squames thick (arrow). SEM. Bar, 200 μm.

epithelial cells was directly related to the degree of stratification of the epithelium. Stratified squamous crypt epithelium contained more glycogen than reticulated epithelium and the more reticulated the patch, the less glycogen it contained. Usually the upper 2–3 cell layers of orderly arranged squames contained glycogen which was absent in the deeper, reticulated cell layers.

Sections stained with PAMB for keratin showed a few mauve–blue squames, occurring either solitarily or in a group, in the top layer of the crypt epithelium. In contrast the stratum corneum of human skin control was always strongly positive. Keratohyalin granules stained blue using the Gram technique and were found in the upper 2–8 epithelial cell layers either in isolated cells or groups of cells.

Both the Alcian blue and the Csaba stain depicted mast cell granules as bright blue deposits encircling the unstained nucleus. Most mast cells were seen in connective tissue areas of the tonsil and a few were occasionally found in reticulated epithelium.

Plasma cells were readily identified using Giemsa's stain. In some patches of reticulated epithelium plasma cells, with abundant purple stained cytoplasm and a clear perinuclear halo, were the predominant cell type, and their perivascular position was conspicuous.

Immunocytochemistry

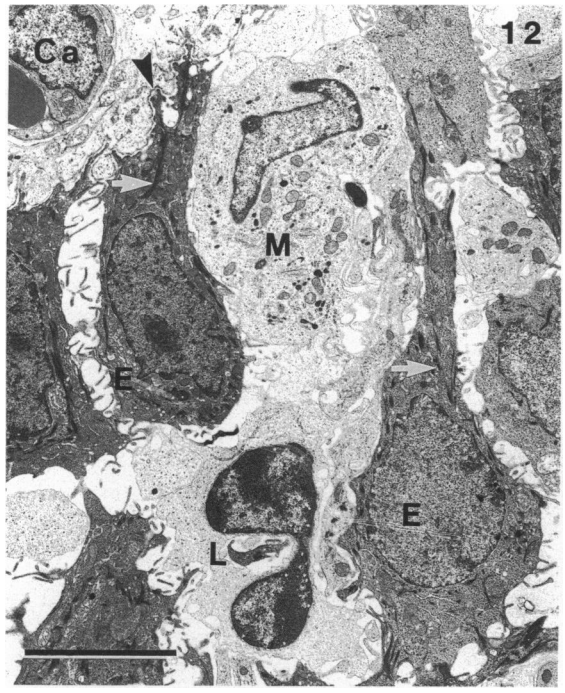
The distribution of CD-3 positive cells in the tonsil was not uniform. The greatest accumulation of T cells was in the interfollicular areas with some in the germinal centres, crypt epithelium and crypt lumina. Most B lymphocytes were confined to the follicular cap regions and a few were seen in the germinal centres, crypt epithelium and crypt lumina (Fig. 8).

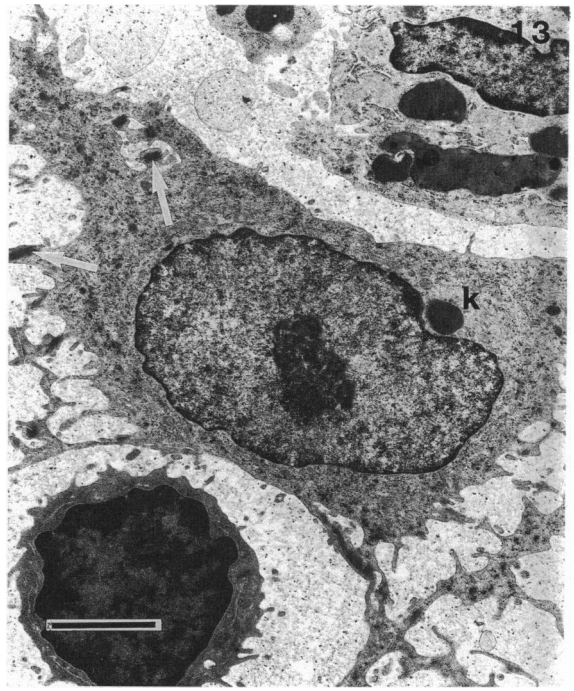
In some patches of crypt epithelium the infiltrating T cells overlay the interfollicular areas, and B cells the summits of the follicular caps, although no such pattern was observed in the patches of heavy reticulation.

The S-100 positive macrophage/interdigitating dendritic cells were mostly seen in the interfollicular T cell regions and in the crypt epithelium above them. They were often surrounded by lymphoid cells (Fig. 9).

Scanning electron microscopy

The crypt surface was mostly composed of intact smooth upper surfaces of closely fitting squames (Fig. 10). When disrupted it contained nonepithelial cells lodged between distorted epithelial cells (Fig. 11) and the transition between an intact and a disrupted





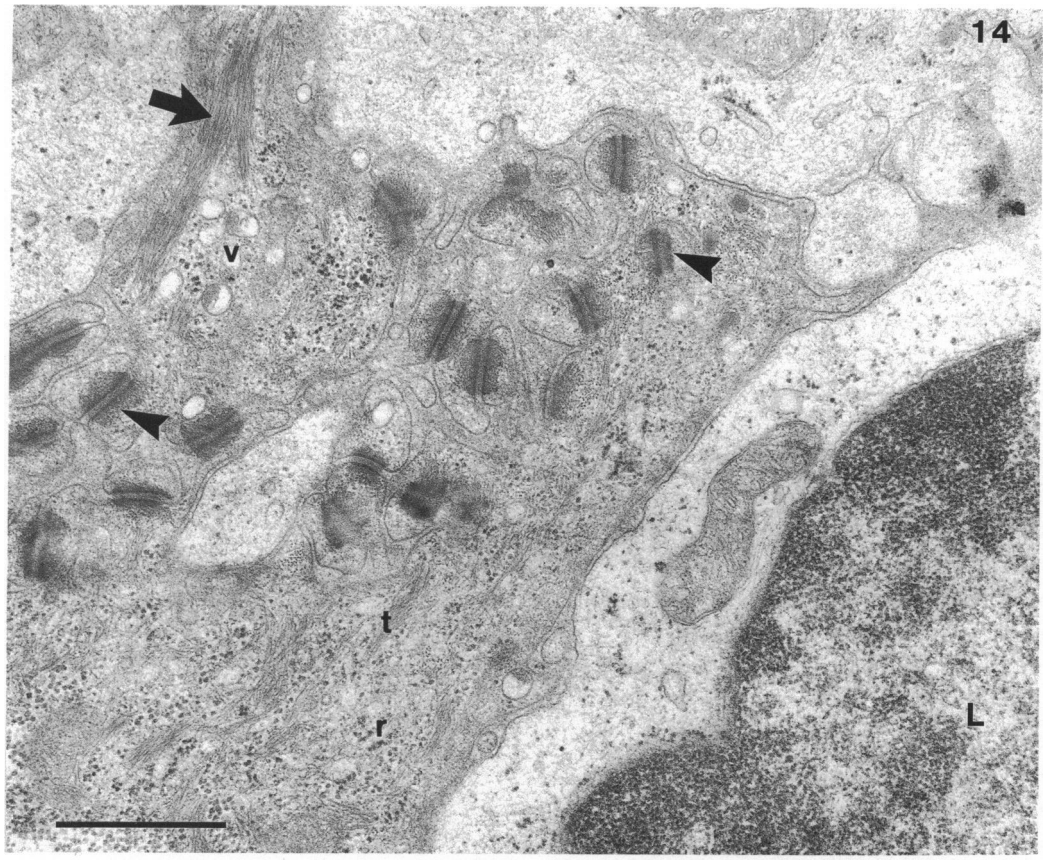


Fig. 12. The electron dense epithelial cells (E) of stratum basale and suprabasale are separated by large intercellular spaces occupied by a macrophage (M) and lymphocyte (L). The epithelial cells possess villi and contain bundles of tonofilaments (arrows). Processes of the basal cells abut on basal lamina (arrowhead) a short distance from a subepithelial capillary (Ca). TEM. Bar, 5 μm.

Fig. 13. An elongated epithelial cell with keratohyalin granule (k) and desmosomes (arrows) surrounded by macrophage (top) and lymphocyte (bottom). TEM. Bar, 2 μm.

Fig. 14. Epithelial cell processes with many desmosomes (arrowheads). Their cytoplasm contains tonofibrils (t), tonofilaments (arrow), ribosomes (r) and membrane-bound vesicles (v). L, lymphocyte. TEM, Bar, 1 μm.

surface was often abrupt. In some areas the crypt epithelium was gently undulating, apparently following the convexity of the underlying follicles (Fig. 10), while in others it exhibited a series of narrow ridges, shallow hollows and small defects. Only a few lymphocytes, erythrocytes and cellular debris lay protected within a crypt.

Transmission electron microscopy

Both epithelial and nonepithelial cells were examined using TEM. Depending on the degree of reticulation, either of these 2 groups of cells could predominate in the reticulated crypt epithelium. In addition, their relationship with the microvasculature was examined.

Epithelial cells

The sectioned basal cells of the reticulated epithelium were cuboidal or cylindrical in shape. They rested on a basement membrane, and were often separated from each other by extensive intercellular spaces (approximately 15–20 μm wide), occupied by numerous nonepithelial cells (Fig. 12) or amorphous intercellular material. Each electron-dense basal cell contained a large oval nucleus with 1–3 nucleoli, numerous free ribosomes, a few profiles of rough endoplasmic reticulum (RER) and a Golgi apparatus, which was frequently associated with smooth membrane-bound vesicles. Aggregates of mitochondria were commonly seen in the infranuclear region. Bundles of short fine tonofilaments constituted an intracellular network arranged mainly in the vertical direction parallel to the long axis of the cells (Fig. 12).

The cell membrane abutted on the undulating basal lamina in short blunted pedicles with numerous small hemidesmosomes. The lateral and apical surfaces of the cells possessed interdigitating villi or folds (Fig. 12), some of which were in desmosomal contact with neighbouring cells. However, in some patches the lateral surfaces of these cells were smooth and in tight apposition.

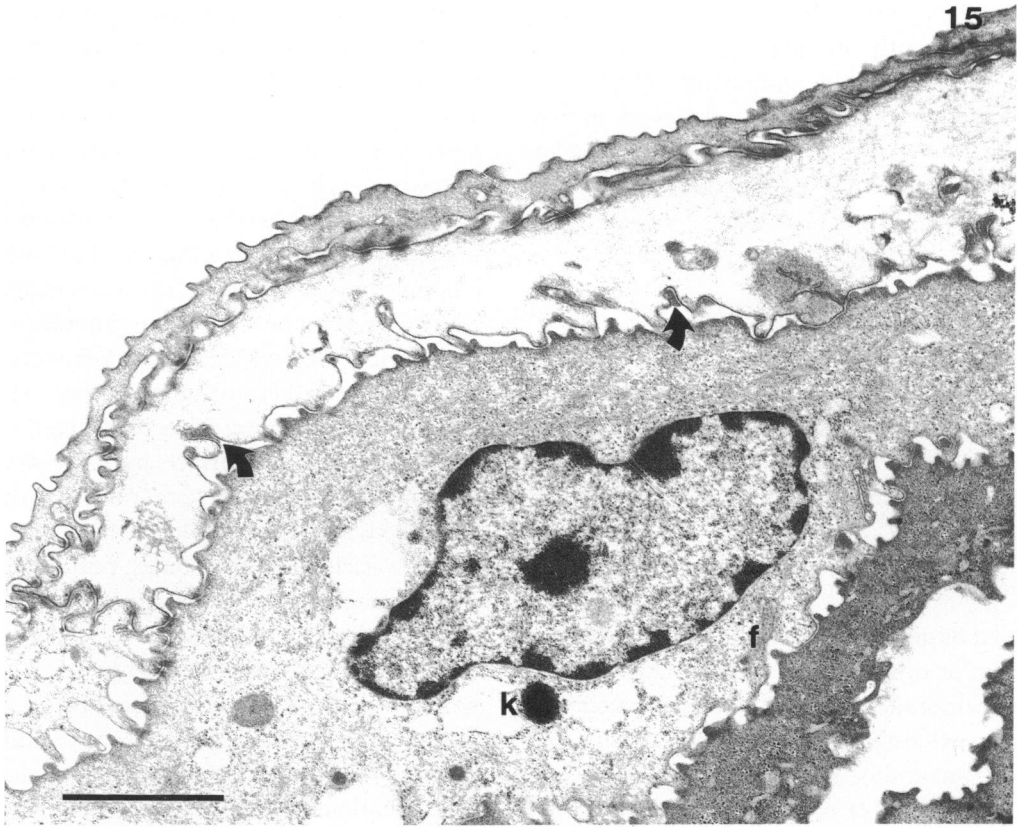
The prickle cell layer of the reticulated epithelium contained cells which were elongated, star, sickle or irregular in shape (Fig. 13). Their many desmosomal connections were of variable length, up to approximately 2 μm, located on the ends of the dendritic processes (Figs 13, 14). The typical features of these cells were the large relatively electron lucent nuclei (6–12 μm in diameter) with 1–3 nucleoli (Fig. 13) and electron dense cytoplasm containing bundles of tonofilaments, numerous ribosomes and membrane-bound pinocytotic vesicles (Fig. 14). Only a few Golgi regions, lysosomes and glycogen storage granules were seen. In some areas these cells also contained keratohyalin granules (Fig. 13).

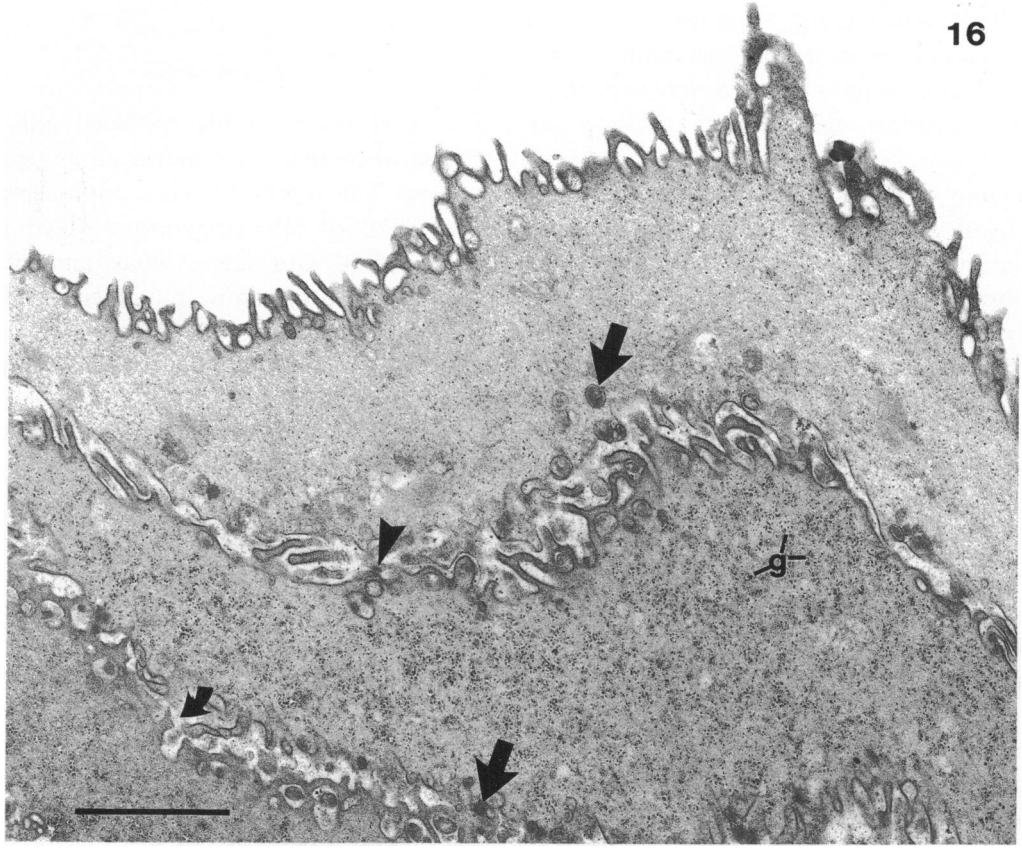
When the superficial cell layer was intact, electron light and electron dark cells were in juxtaposition, following each other to the epithelial surface in no particular sequence (Fig. 15). Usually these cells in the upper layers possessed a thickened cell membrane and contained pyknotic nuclei, cytoplasmic filaments, free ribosomes, membrane-coating granules of the dense-core variety, aggregates of glycogen storage granules, and sometimes keratohyalin granules (Figs 15, 16). Other areas were covered by only 1 epithelial cell with a convex proximal surface bearing short microvilli (Fig. 17). These cells were rich in organelles and contained a healthy nucleus, numerous micropinocytotic vesicles, free ribosomes, polyribosomes, RER, a few mitochondria, Golgi apparatus and sometimes keratohyalin granules (Fig. 17).

In areas where the top cells had sloughed off, the remaining epithelium was covered by only a thin layer of superficial cells. Sometimes, this process of desquamation could remove all the superficial cells and in such situations, the surface was denuded and appeared disrupted, consisting of elongated and distorted epithelial cells surrounded by many nonepithelial cells.

Infiltrating nonepithelial cells

The morphology of the epithelial cells was often altered where there were numerous nonepithelial cells present. The following types of nonepithelial cells were identified. (1) Lymphocytes of various morphologies often with microvillous and pseudopodial cytoplasmic projections, found either singly or in groups (Figs 12–14). Clusters of lymphocytes were sometimes tightly packed in cavernous hollows of the epithelial cell network (Fig. 17). (2) Plasma cells, in perivascular locations (Fig. 18) or scattered through the thickness of the reticulated epithelium. (3) Macrophages, with their characteristic organelles (Figs 13, 19), possessing numerous processes extending over a large epithelial territory (Figs 12, 19). (4) Interdigitating cells, containing large, bizarre-shaped nuclei surrounded by moderately electron dense cytoplasm (Fig. 19). No Langerhans cell granules were found in any of these cells. (5) Free extravasated erythrocytes, either isolated or in small groups, lying between the epithelial cells. They were not always in the immediate vicinity of a capillary. (6) A few polymorphonuclear leucocytes, mostly neutrophils, in the superficial layers of the epithelium. (7) Rare mast cells.





Figs 15, 16. For legend see opposite.

Relationship between capillaries, HEVs and the reticulated epithelium

The vessels in subepithelial locations, with their associated pericytes, were usually separated from the overlying basement membrane of the epithelium by a varying amount of connective tissue (Fig. 12), which in places also contained a lymphocyte or macrophage. Sometimes only a very thin layer (0.2–0.5 μm) of collagen or amorphous connective tissue intervened between the epithelial basement membrane and the underlying capillary wall. Often the endothelial lining of a capillary exhibited fenestrations on the aspect of its wall facing the epithelium (Fig. 20).

Capillaries situated within connective tissue papillae were surrounded by perivascular spaces, which contained numerous lymphocytes and plasma cells (Fig. 18). These papillae were demarcated from the surrounding epithelium by a mostly intact basement membrane.

Both the capillaries and HEVs in an intraepithelial location were surrounded by pericytes. External to them, and in close circumferential contact, lay the epithelial and nonepithelial cells. In this situation the epithelial basement membrane was often indistinct or apparently absent (Fig. 21).

DISCUSSION

The crypt epithelium of the palatine tonsils was reticulated in all the subjects examined. It was a modified form of stratified squamous epithelium, which contained lymphocytes, plasma cells, macrophages and interdigitating cells. These cell types are known to be present in the crypt epithelium as early as the 15th week of gestation (von Gaudecker & Müller-Hermelink 1982*a*), which suggests that reticulation is a normal developmental event, and occurs concurrently with the formation of primary follicles, interfollicular areas and HEVs (von Gaudecker & Müller-Hermelink, 1982*b*). Reticulation continues throughout life and the presence of nonepithelial cells is considered a physiological characteristic of this epithelium, which is constantly exposed to air-borne and alimentary antigens. Any inflammatory changes are superimposed on the pattern of reticulation (Falk & Mootz, 1973). In the present study polymorphonuclear leucocytes were absent or few, indicating that there was no acute inflammation in the subjects' tonsils.

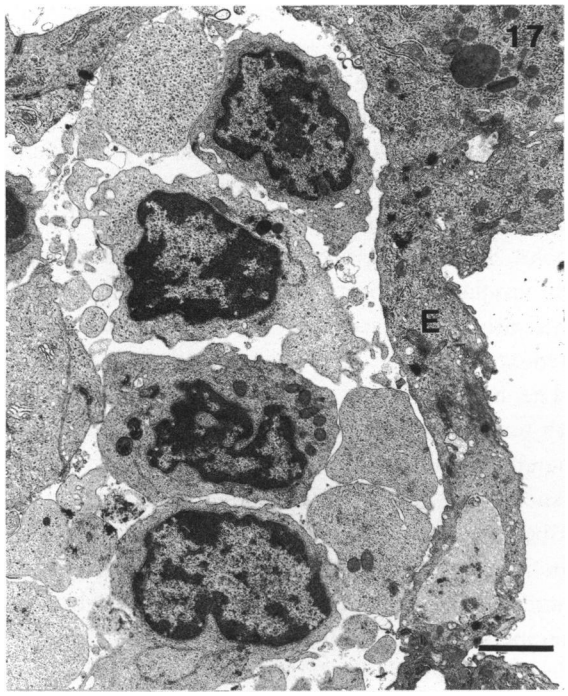
The main structural features of the crypt epithelium were, first, the epithelial cells, altered in shape and cellular contents but still representing its building blocks; second, the presence, and in some areas the predominance, of nonepithelial cells; and third, the intraepithelial location of numerous small vessels, often extending throughout the epithelial thickness.

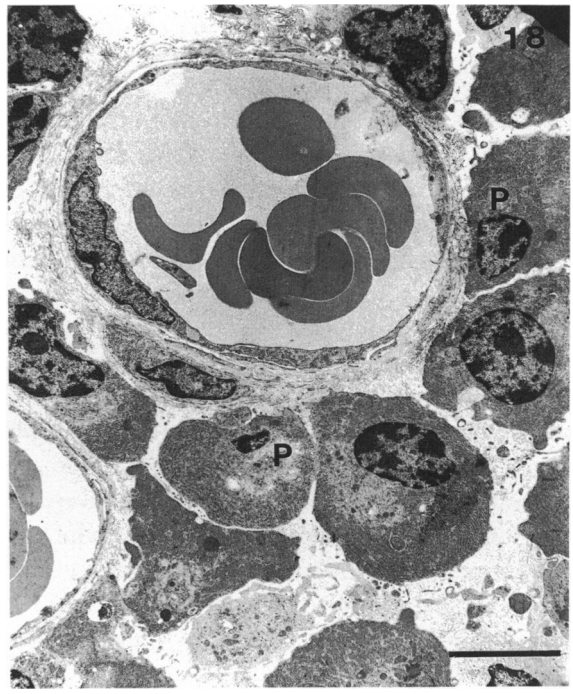
The infiltration of the tonsillar crypt epithelium with nonepithelial cells was not uniform, resulting in a patchy reticulated appearance. When it was modified to such a degree that the characteristics ascribed to an epithelium were lost, it presented the appearance of a functional lymphoid compartment (Fioretti, 1957; Brandtzaeg, 1988). These reticulated patches were interspersed with patches of stratified squamous epithelium, which contained only a few infiltrating cells. Presumably the sites of the reticulated patches in any individual tonsil were not predetermined, but varied with time and depended on the degree of the current antigenic stimulation. Hence, the structure of the palatine tonsil as a whole, and especially that of the sponge-like reticulated epithelium, render a direct transport of foreign material from the external environment of the oropharynx to the tonsillar lymphoid cells possible. The direct transepithelial access of antigens may, in turn, result in greater influx and recruitment of nonepithelial cells in a particular patch, in contrast to a less strongly stimulated area which may have retained stratified squamous nonkeratinised cover.

The system of crypts increases considerably the total epithelial surface area of the tonsil (Kassay & Sandor, 1962; Oláh, 1978; Howie, 1980). Slipka & Kotyza (1987) estimated that in an 'average' human palatine tonsil the epithelial surface area of the crypts measured 295 cm^2, in addition to the 45 cm^2 of the epithelium covering the oropharyngeal surface. The dynamic nature of the tonsillar lymphoid tissue, such as changes in number and sizes of follicles, may account for the varied shapes and sizes of crypts. In their computerised 3-dimensional reconstruction of the crypt system Abbey & Kawabata (1988) showed

Fig. 15. Juxtaposition of electron light, moderately electron dense and electron dense epithelial cells with thickened cell membranes and blunted microvilli on the epithelial surface. Intercellular electron dense material (arrows) is located between the layers. Note the keratohyaline granule (k) and cytoplasmic filaments (f). TEM. Bar, 2 μm.

Fig. 16. A patch of stratified squamous nonkeratinising crypt epithelium with elongated microvilli on the surface. There are only a few small desmosomes (arrowhead). Electron dense and fine granular material is seen in the intercellular spaces (curved arrow). Glycogen granules (g) and membrane coated granules (straight arrows) contribute to the cytoplasmic contents. TEM. Bar, 2 μm.





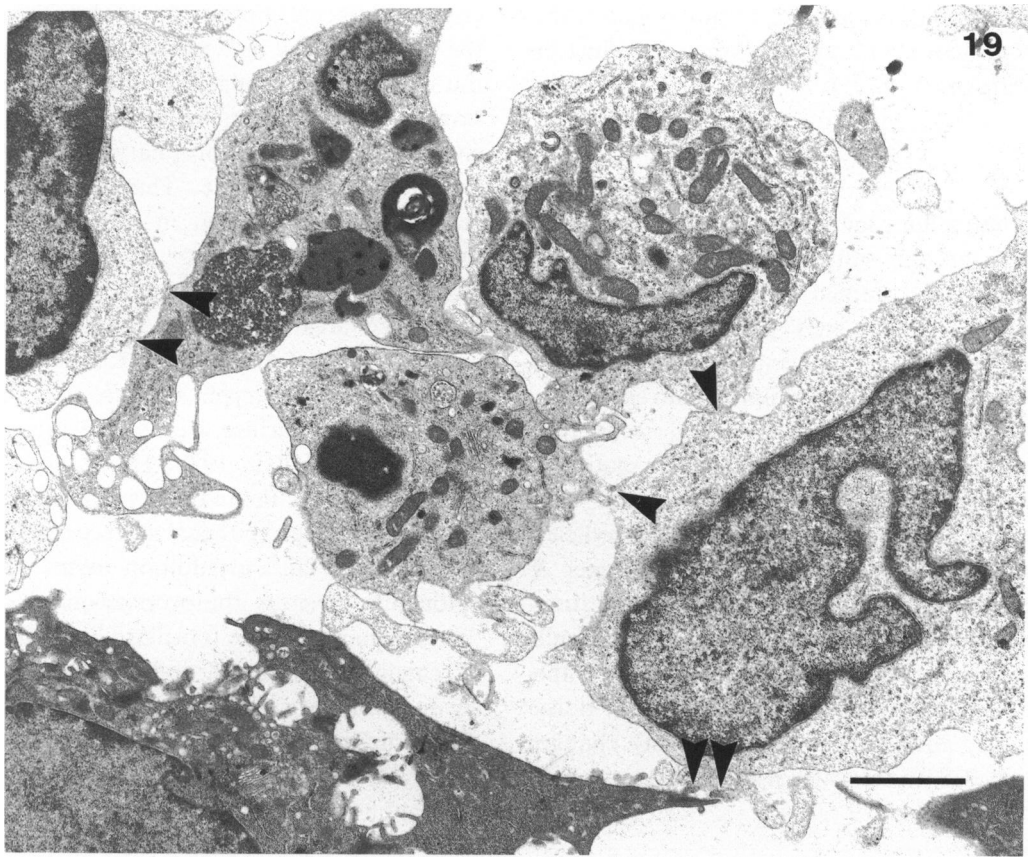


Fig. 17. Reticulated crypt epithelium showing a nest of 4 infiltrating lymphocytes (middle) covered by epithelial cells (E) containing numerous cytoplasmic organelles. TEM. Bar, 2 μm.

Fig. 18. Intraepithelial capillaries in a finger-like projection of subepithelial connective tissue surrounded by large plasma cells (P). TEM. Bar, 5 μm.

Fig. 19. An area of reticulated epithelium showing three macrophages (centre), an interdigitating cell with a bizarre-shaped electron-dense nucleus (right), a lymphocyte (left) and an epithelial cell (bottom). Intimate contacts between these cells are indicated by arrowheads. TEM. Bar, 2 μm.

that the crypts had not only branches but also anastomoses. Together with the changes in follicular sizes the arborisation of crypts is a likely reason for variations in the contours of crypt epithelium when viewed in the present study with SEM. Degenerating epithelial and nonepithelial cells, together with their liberated organelles and cellular debris, became the contents of the crypts. Moreover, the reticulation of the palatine and thymic epithelium seems always to be accompanied by degeneration of epithelial cells and formation of epithelial corpuscles (Slipka & Kotyza, 1987; Perry & Slipka, 1993).

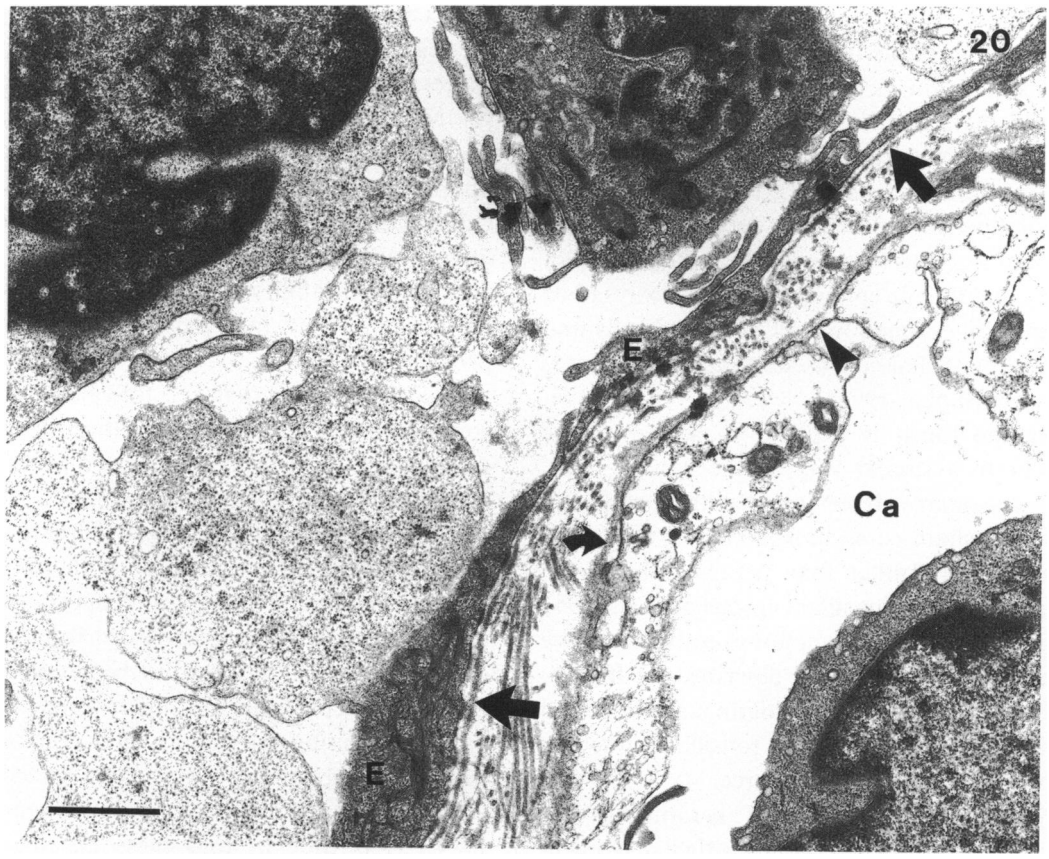
The palatine tonsil is exposed to a hydrophilic environment in a similar way as the human fetus, hence it is reasonable to suggest that their epithelial coverings may share some morphological and functional similarities. Rather than acting as a passive protective layer the tonsillar epithelial cells may exhibit energy-demanding functions, both absorptive and secretory, similar to those described by Breathnach (1971, 1981) in the fetal periderm, and the stored glycogen in the upper strata of the tonsillar epithelium may form the required energy source.

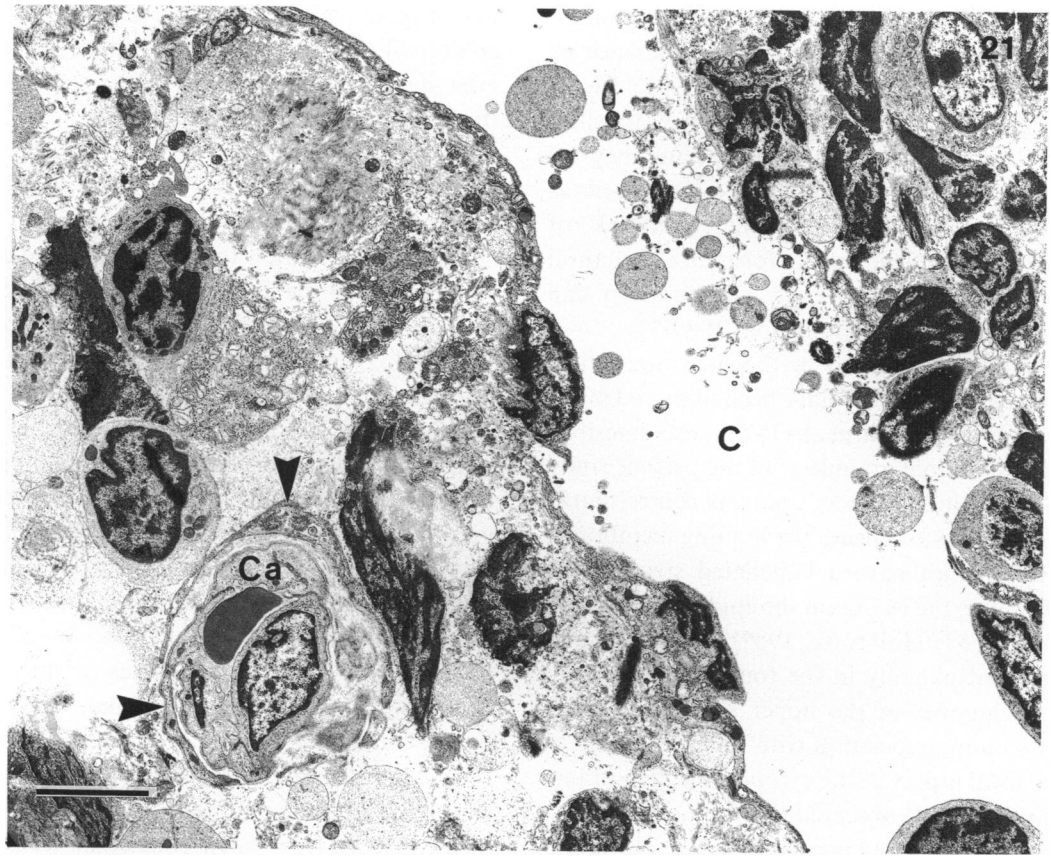
The cytoplasmic organelles of keratinised and nonkeratinised stratified squamous epithelia belong to a common group, which includes tonofilaments, membrane-coating granules (MCGs) and keratohyalin granules (KGs). However, the ultrastructure of these organelles varies according to the presence or absence of keratinisation (Schroeder, 1981; Landmann, 1986; Holbrook, 1989). In the tonsillar epithelium the tonofilaments were diffuse and they did not always form conspicuous bundles of tonofibrils as in the skin, where an interlacing meshwork of tonofibrils is a prominent feature forming a structural framework on which events of keratinisation can occur (Breathnach, 1971; Holbrook, 1989).

The only reference found in the literature where membrane-coating granules have been observed in the palatine tonsil was by Oláh et al. (1975), who described them as 'phospholipid granules'. In the present study the MGCs contained a finely granular central dark-core with a clear zone beneath the limiting membrane, and usually did not have a lamellated structure as found typically in the MCGs in the epidermis (Odland & Holbrook, 1981; Holbrook, 1989). These granules were distributed patchily in the tonsillar epithelium, increased in number in the upper strata, and were frequently seen in association with Golgi complex as well as the distal aspect of the cytoplasmic membrane. Chen & Squier (1984) observed that in the epidermis, cell membrane thickening occurred several cell layers above the areas where MCGs were expelled into the intercellular spaces. Such events may account for the presence of fine granular material in the intercellular spaces seen in some patches of the tonsillar epithelium. As MCGs are thought to contain phospholipids (Breathnach, 1971; Hayward, 1979), glycoproteins, lipoproteins and lysosomal enzymes (Hayward, 1979; Odland & Holbrook, 1981) they, together with the intercellular material, may contribute not only to an effective permeability barrier, as recognised in the skin (Holbrook, 1989), but also to the tonsillar mucosal protection.

It is well known that keratohyalin granules show considerable morphological variation among different species and even among topographically related keratinising epithelia (Jessen, 1970; Breathnach, 1977). In the tonsillar crypt epithelium the KGs were either single, dense, associated with ribosomes and possibly membrane bound, or composite, arranged as groups of single dense granules surrounded by a less dense matrix associated with ribosomes. In contrast, KGs in the epidermis are highly irregular in shape, have no limiting membrane, and contain electron-dense material intimately associated with surrounding tonofilaments (Breathnach, 1971; Chen & Squier, 1984). It is thought that these granules may also contribute to the thickening of the plasma membrane (Chen & Squier, 1984) or that they may provide matrix that embeds keratin filaments of cornified epidermal cells (Stenn, 1983; Holbrook, 1989). However, despite the thickening of the cell membrane in the nonkeratinising tonsillar crypt epithelium the KGs were not widely distributed, thus the membrane thickening may originate from the expelled MCGs rather than the intracellular KGs.

Lymphocytes often infiltrated the whole thickness of the crypt epithelium with no distinct patterns of B and T cell distribution. These cells presumably migrated from the subepithelial lymphoid tissue through the connective tissue and the basement membrane to reach the epithelium, although some circulating cells might have extravasated directly into this location through the walls of the intraepithelial vessels (Perry et al. 1988, 1991). Perivascular lymphocytes and numerous plasma cells, capable of local production of immunoglobulins (Maeda et al. 1982), were found around these vessels. In addition, the subepithelially located capillaries, with fenestrations in the walls facing the epithelial basement membrane, contained micropinocytotic vesicles and multivesicular bodies in the endothelial cell cytoplasm. This suggests that these endothelial cells could participate in the transport of water soluble molecules, such as circulating immunoglobulins, across the capillary wall





Figs 20, 21. For legend see opposite.

into the intercellular tissue spaces, and add to the pool of immunoglobulins permeating the reticulated epithelium.

The distribution of S-100 positive cells in the reticulated crypt epithelium was patchy. The ultrastructural findings provided morphological evidence to support the concept that monocyte-macrophage and dendritic cells cooperate closely in the processing and presentation of foreign antigens to the tonsillar lymphocytes (Yamamoto et al. 1992). Thus one of the major functions of reticulated epithelium may be to provide favourable environment for the contact between intraepithelial lymphocytes, antigen presenting cells and antigens.

The right balance of the numbers of epithelial and nonepithelial cells in the reticulated crypt epithelium might be vital for the preservation of efficient mucosal protection. The locally produced secretory IgA (sIgA) molecule is stabilised and transported by the secretory component in the cytoplasmic membrane of epithelial cells, which synthesise this polypeptide (Brandtzaeg, 1984; König et al. 1987). This transport is unique in that the secretory component is not recycled, but must be continually resynthesised by the epithelial cells. Thus a sufficient number of competent epithelial cells must be present in a reticulated patch in order to retain this function.

In some patches the infiltrating cells almost reached the uppermost stratum and the epithelial surface, provided by only 1 or 2 cell layers, remained intact. This type of epithelium, described by Fioretti (1957) as 'the lympho-epithelial symbiosis', acquired the appearance and presumably also the role of a lymphoid compartment and lost many of its classic epithelial characteristics. However, it still maintained a cover and thus protected the 'internal environment' of the tonsil from the 'external environment' of the crypt lumen.

In response to the mobile invading cells the accommodating stratified squamous cells assumed bizarre shapes, accompanied by a rearrangement of their cytoskeleton of tonofilaments, an apparent increase in ribosomes and mitochondria, and by redistribution of desmosomal contacts. Hence the lymphocytic infiltration probably induces changes in the expression of keratin in epithelial cells (Sato et al. 1988), which in turn initiate the transformation of epithelial cells from stratified squamous into star-shaped. The properties of the keratin intermediate filament network, attached to cell surface adhesion complexes, i.e. desmosomes and hemidesmosomes, are dynamic (Coulombe, 1993). Desmosomes may be modulated during epithelial morphogenesis and the extent of desmosomal adhesion may be affected by external signals: transforming growth factor β can increase expression of desmosomes in epithelial cells, while the reduction in extracellular Ca^{2+} concentration can disrupt the initial desmosomal adhesion mediated by the calcium-dependent cadherin family of adhesion molecules (Garrod, 1993). In order to facilitate cell motility, the modulation of cell-substratum adhesion is reflected in the dynamic structure of hemidesmosomes. They contain the α6β4 integrin complex (Garrod, 1993), which may play a key role in signal transduction not only for epidermal, but also for epithelial stratification, differentiation and basal cell proliferation at sites such as the palatine tonsil. Further studies, using immunoelectron microscopy, are planned to elucidate the role of desmosomes and hemidesmosomes in tonsillar epithelium.

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Fig. 20. Subepithelial capillary (Ca) showing fenestrations in its wall (arrowhead). A thin layer of collagen separates the basal lamina of the endothelial cells (curved arrow) from that of the epithelial cells (straight arrows). Note the attenuated epithelial cell processes (E). TEM. Bar, 1 μm.

Fig. 21. Tonsillar crypt (C) lined by degenerating and desquamating reticulated epithelium containing an intraepithelial capillary (Ca). It is surrounded by pericytes (arrowheads) and the lumen is occupied by an erythrocyte. TEM. Bar, 5 μm.

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